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THE MICROBIAL COMMUNITY IN THE LAYERED SEDIMENTS AT LAGUNA FIGUEROA, BAJA CALIFORNIA, MEXICO: DOES IT HAVE PRECAMBRIAN ANALOGUES?

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ABSTRACT

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In the hypersaline lagoon at Laguna Figueroa vertically stratified diverse communities of microorganisms thrive. The modern sediments of Baja California at Laguna Figueroa contain cyanobacterial communities and sedimentary structures produced by these blue greens that have already been studied by Horodyski and his colleagues. This paper provides an introduction to the complex microbial communities, primarily those that underlie the laminated *Microcoleus* mats. They are composed of anaerobic photosynthetic and heterotrophic bacteria.

The following genera of cyanobacteria at least are components of these mat communities: *Lyngbya, Microcoleus, Entophysalis, Phormidium, Pseudoanabaena, Anabaena and Schizothrix.* Among the photosynthetic bacteria several species of *Thiocapsa*-like microbes formed major surface components of certain mats and scums; rhodospirilli, rhodopseudomonads, chromatis and others were seen.

The following nonphotosynthetic bacteria were identified: *Nocardia* sp., three types of spirilli, two types of *Spirochaeta* sp., two types of *Desulfovibria* sp., a new strain of red *Beneckea* and four distinctive unidentified coccoid and filamentous bacteria. Reasons are given for believing several of the species are new to science and that the microbial diversity is far greater than the approximately twenty species reported here. Eukaryotes are extreme ly rare. Only one species of animal, a herpachtechoid copepod, was ever seen in the 8-km long microbial communities of the hypersaline basin. *Dunaliella salina,* a chlorophyte and *Aspergillus sydowi,* an ascomycetous fungus were the only eukaryotes that were observed to be regular components of mat communities. Ciliates, amoebae (including a chrysarchnion-like microbe) and diatom tests, mostly empty, were the only other eukaryotes observed. Attempts to enrich for eukaryotic microorganisms were not successful whereas attempts to enrich for bacteria, especially anaerobes led to such a profusion of forms that to continue detailed study of them was beyond our means. Unidentified small rods and cocci constituted the largest fraction of individuals in the subsurface community. The microbes isolated from mats are adapted for alternating dry and wet conditions as well as high concentrations of salt and low concentrations of oxygen.

The shales and cherts of the Swartkoppie Zone of the Swaziland System near Barberton, South Africa dated as older than 3400 Ma, are well-preserved Archean sediments in which significant quantities of both reduced carbon and structures interpreted to be microfossils have been found. Although further detailed study will be required, it appears likely to us that the layered communities of microorganisms growing in the semi-tropical hypersaline lagoon in Baja California, Mexico, provide possible analogues to some Precambrian sediments and thus that anaerobic microbial communities were already well developed by 3400 Ma ago.

INTRODUCTION

To most fully interpret the Precambrian fossil record, available as stromatolites, algal mats and microfossils, modern analogues are studied for comparison (Golubic, 1973; Walter, 1976). Studies of this type emphasize the cyanobacterial community, omitting the less conspicious bacterial components. This paper, the first in a series of comparisons between fossilized and living sediments, introduces the bacterial diversity of the Laguna basin. A detailed study of a new red *Beneckea* has already been undertaken (Giovannoni, 1979, 1980).

Laguna Figueroa (= Laguna Mormona), Baja California del Norte, Mexico, is a closed hypersaline lagoonal complex consisting of an evaporite flat and salt marsh. It extends some 16 × 2½ km during wetter periods, with higher areas always exposed. The high rate of evaporation in this semi-desert region and the influx of seawater through the barrier dune ridge separating the lagoon from the sea contribute to the development of sedimentary structures reminiscent of Precambrian stromatolites (Horodyski et al., 1977).

Three major genera of cyanobacteria, *Entophysalis*, *Lyngbya*, and *Microcoleus* (blue green algae, Stanier and Cohen-Bazire, 1977), respectively dominate in the formation of the three morphologically distinct microbial mat types found at this site. Their community structure, degradation patterns, and involvement in sedimentation processes have been elucidated by Horodyski and Van der Haar (1975), Horodyski (1977), and Horodyski et al. (1977). The most recent publication from this group claims "this paper completes the study of the Laguna Mormona mats and concerns itself with the *Lyngbya aestuarii* mats, associated tufted cyanophytic mats, and their comparison with superficially similar Precambrian structures." (Horodyski et al., 1977, p. 1305). However, their studies did not include bacteria, particularly the photosynthetic and other anaerobes as well as many other microbes of importance to mat formation and change. This paper presents an introduction to other members of the microbial community associated primarily with the low, flat mat, dominated by *Microcoleus*.

We were initially impressed by the striking similarity in appearance between a 3400 Ma thinly laminated chert taken from the Swartkoppie facies of the upper Onverwacht (or lowermost Fig Tree) Group of the Swaziland Sequence of South Africa and typical sections taken from any of the drier sites through the *Microcoleus* mat surface at Laguna Figueroa (as shown in Figs. 1 and 2). Subsequent visits to the site (May, 1977; August, 1977; and August, 1978) reaffirmed our initial impression that there might be a parallel between

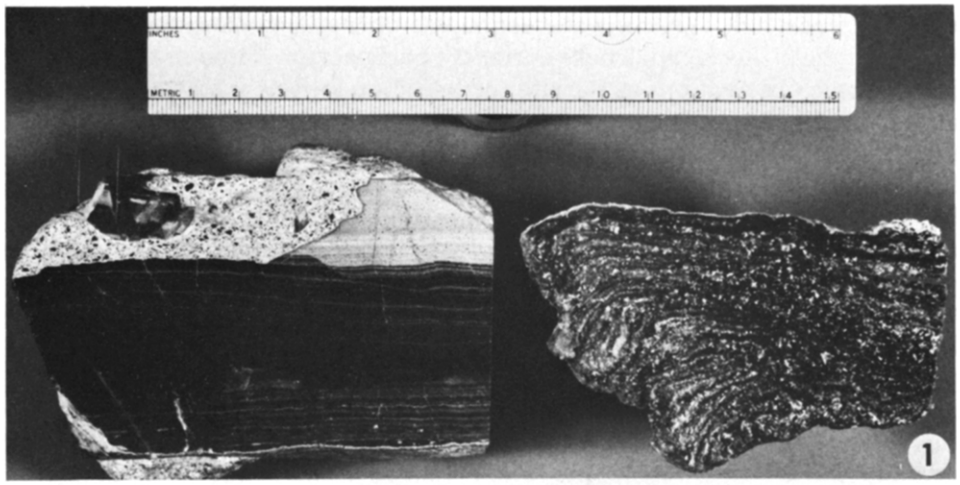
certain carbon-rich, shallow-water sediments of the Early Precambrian (that is, the Archean Aeon) and those currently being accumulated in the lagoon. Microscopic observations at the site further impressed us with the abundance and diversity of microorganisms other than cyanobacteria. The first few millimeters of evaporitic sediment at the basin contains large populations of live, intertwined cyanobacteria. We have found the microbial-mat community to be far more extensive than surface communities dominated by *Microcoleus*, *Entophysalis*, or *Lyngbya*, as reported by the Horodyski group. From samples collected at various locations we have isolated viable anaerobic and facultatively anaerobic bacteria from samples taken at depths of at least 30 cm, the level at which, in some locations, one can see the mineralized paleosurface of the flat mat that once was dominated by the cyanobacterium *Microcoleus*.

Our interest is in comparing these live communities with certain carbon-rich laminated sediments from the Swaziland Sequence. We are particularly concerned with the Swartkoppie "Zone" between the Onverwacht Group and the immediately overlying Sheba Formation (average 700 m thick), the lowermost of the Fig Tree Group. The shallow-water sediments now forming at Laguna Figueroa seem comparable to thinly laminated cherts of limited distribution in the Swartkoppie.

The earliest convincing evidence of a widespread and biologically productive ecosystem now known is from this thick column of diverse sediments, volcanics and conglomerates comprising the Swaziland Sequence of the eastern Transvaal of the Republic of South Africa and adjacent Swaziland. The basal volcanic units are dated at about $3.4—3.5 \cdot 10^9$ years (Jahn and Shih, 1974). Certain sedimentary facies, in particular the black cherts, contain evidence of structurally preserved microorganisms. In addition, the geochemistry of the stable carbon and sulfur isotopes, yield putative evidence of biological fractionation through biochemical pathways which are known in extant living systems to select for the "lighter" stable isotopes of these two elements (Perry et al., 1971; Eichmann and Schidlowski, 1975). Moreover there are present locally abundant banded iron formation and jasperized chert beds from which it can be inferred that microbial oxygenic photosynthesis in localized environments had evolved.

The Swaziland Sequence is an isolated block of supracrustal rocks consisting of interbedded sediments, volcanics and other igneous rocks in which are intercalated organic-rich cherts and shales indicative of shallow-water subsiding basinal deposition. The entire Sequence was injected and uplifted from below by granitic bodies, some of which are dated at about 3 Ga. Fortuitously the tectonic processes attending uplift of the Swaziland Sequence resulted in mineral thermal alteration ($<275°C$) since deposition, and thus the sedimentary units are remarkable well preserved. In a sense, the entire System is a complex of superimposed rock units floated on a sea of granite with much of its lithologic integrity preserved and decipherable, highly altered only where it is in contact with igneous intrusives.

The Swaziland Sequence is essentially a conformable series of both sediments and igneous rocks but divisible lithologically into three groups of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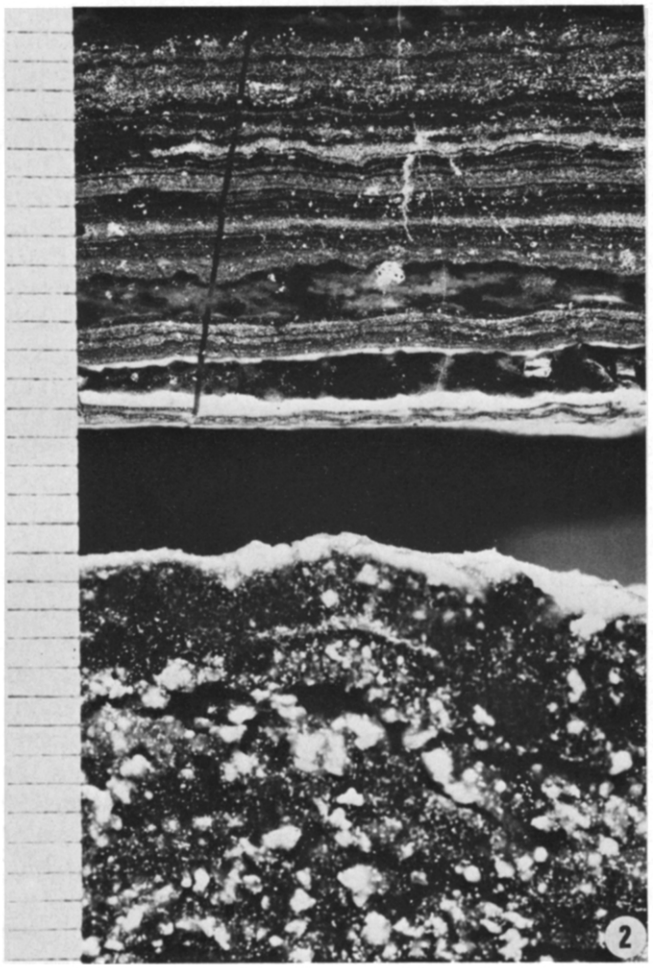
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Formations: (1) the lowermost Onverwacht Group (averaging 15,230 m in thickness), (2) the Fig Tree Group (averaging 2150 m), and (3) the Moodies Group (averaging 3150 m). It is possible that the entire Swaziland Sequence was deposited within a time span of 200—300 Ma although inhomogeneities of rock types in the Moodies Group conglomerates preclude delineating the time precisely.

The most convincing evidence of microorganisms comprising three distinct size classes of fairly well defined modal distribution occur in cherts in the Swartkoppie "Zone" transitional between the uppermost Onverwacht and the basal Fig Tree Groups. During the past decade and from various laboratories, more than a dozen reports have been published describing microfossils and the organic biogeochemistry from chert units of both the Onverwacht and the Fig Tree cherts (Knoll and Barghoorn, 1977; Muir, 1978; Nagy and Nagy, 1968). Extensive microfossil and chemical data have been compiled by Reimer (1975).

In terms of sedimentary and geochemical manifestation of an Archean biosphere and ecosystem, the shales and interbedded graywackes of the Sheba Formation present a virtually unique opportunity to quantitate even approximately the biological productivity of an Archean basin. The Sheba Formation has been intensively studied both stratigraphically and geochemically, in part because of its gold-bearing units in the carbonaceous shales. Sedimentologically the original basin was about 100 × 40 km with an average thickness of 700 m. On the basis of detailed data on the known average reduced carbon values the basin contains about $52 \cdot 10^9$ tons of carbon. Assuming a duration of sedimentation of $5 \cdot 10^9$ years these data work out to an average preserved carbon content of 0.32 g of carbon per year per m^2 (Reimer et al., 1979). It is doubtful if much, if any, of the Sheba Foundation is of abiological origin in view of the stable carbon isotope ratios which are indicative of biogenicity ($\delta^{13}C$ $-25^0/_{00}$ to $-30^0/_{00}$, Eichmann and Schidlowski, 1975) and also because of the sedimentological distribution of the reduced carbon (Reimer et al., 1979).

We plan to make direct measurements of rates of primary productivity by the layered anaerobic photosynthesizers at Laguna Figueroa as well as the rates of preservation of this organic carbon in order to compare extant and fossil bacterial ecosystems.

This paper, the first in a series of comparisons between these fossilized and living sediments, describes the bacterial diversity of the Baja basin as a backdrop to several detailed studies of individual groups of indigenous microbes, for example, a new *Beneckea* (Giovannoni, 1979, 1980).

Fig. 1. Left: Banded chert from the abandoned Montrose gold mine, Swartkoppie facies, Barberton mountain land, collected in 1972 by E.S. Barghoorn. Right: Upper surface of sediment taken from flat mat dominated by *Microcoleus*, August, 1977. Laguna Figueroa. Top scale inches, bottom in mm.

Fig. 2. Top: 3400 Ma old banded chert. Bottom: microbial mat. Same as Fig. 1 but at higher magnification. Photo courtesy of Wm. Ormerod. Scale in mm.

Because of the extreme conditions of salinity, desiccation, and high temperatures there are very few animals or higher plants in the area of the mats. In fact, with the exception of a green flagellate, *Enteromorpha*, diatom tests, a single species of fungus, and herpachtechoid copepods, eukaryotic organisms were rarely seen.

Our hypothesis that this lagoonal basin may prove a modern analogue for certain aspects of the Archean carbon-rich lithologies is supported by the prevailing anaerobiosis, the absence of plants and the presence of extensive populations of photosynthetic microorganisms that deposit carbon-rich sediment layers. In further support of this hypothesis we present here brief descriptions of some of the major types of microbes we have observed, either by light or electron microscopy, in samples collected in the field (Aug. 1977 and Aug. 1978). Included are only those that were morphologically distinctive or were easily isolated into pure cultures from anaerobic enrichments. The total microbial diversity is, of course, still not completely known, but even our preliminary observations indicate that it is incredibly more complex than reported in this paper.

FIELD SITES

The extensive Laguna Figueroa salt flat near the Pacific Ocean — known locally as "las salinas" — is about 250 km south of San Diego and about 15

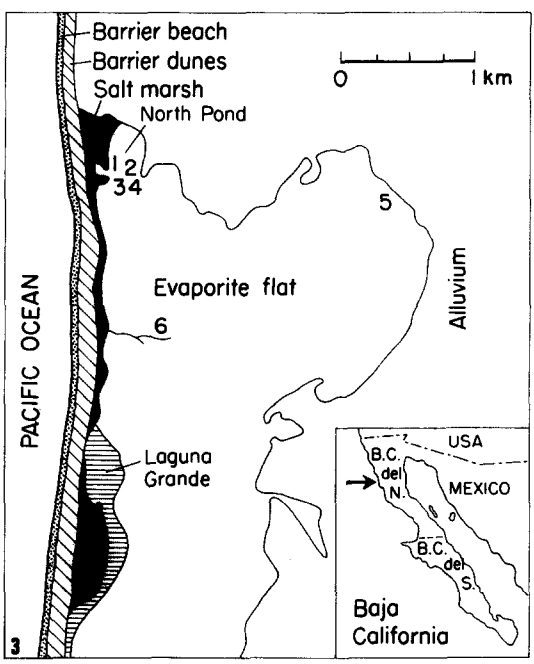


Fig. 3. Sketch map of Laguna Figueroa showing locations of sites as described in the text (modified after R. Horodyski).

km north of San Quintin. Our observations were taken at the north end of the salt flat near the clam camp, with samples collected at the six locations noted in Fig. 3. A brief description of each site follows:

Site 1. A flat mat dominated by the cyanobacterium *Microcoleus* (probably) *cthonoplastes*, R.J. Horodyski (1978, pers. comm.). Characteristic of this site are depressions made by tire tracks and footprints and subsequently filled with very red lagoon water (Fig. 4).

Site 2. Pans of white foamy water surrounded by large evaporite crystals primarily pink gypsum and halite. The higher portions of the *Microcoleus* mat tends to form such desiccation polygons (Horodyski et al., 1977) (Fig. 5).

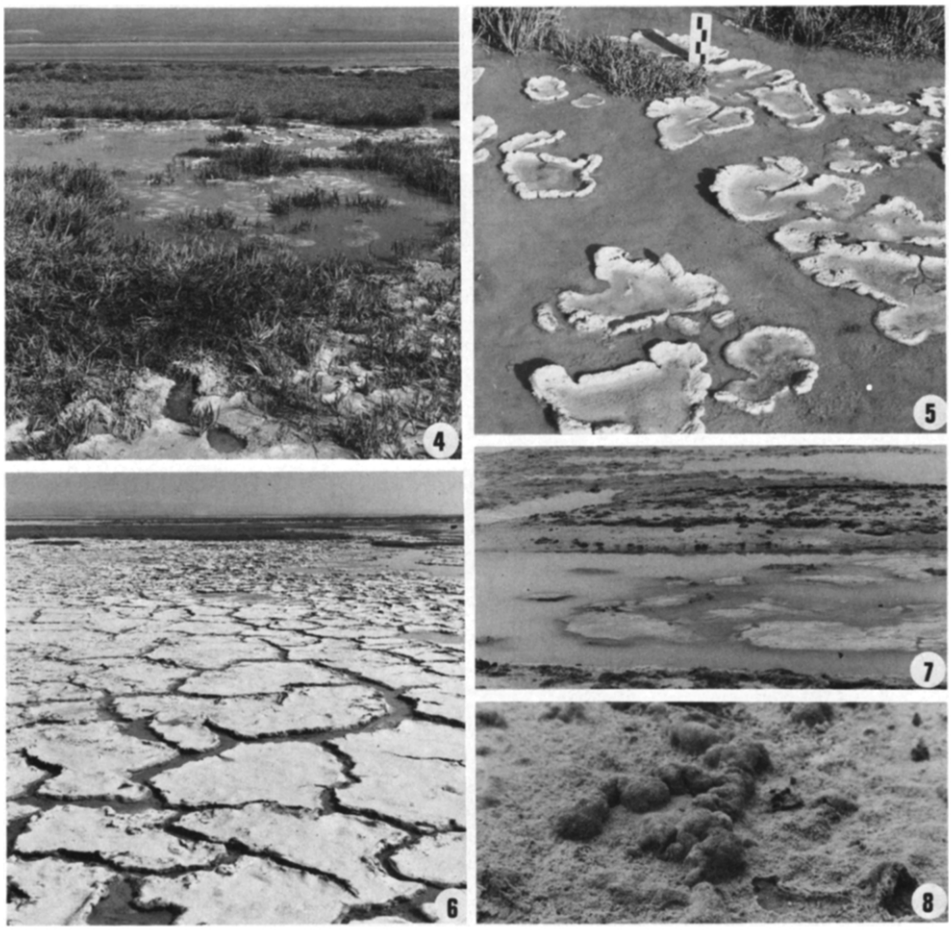


Fig. 4. North Lagoon, site 1. Photo courtesy of R. Horodyski.
Fig. 5. North Lagoon, site 2. Photo courtesy of R. Horodyski.
Fig. 6. North Lagoon, site 3. Photo courtesy of R. Horodyski.
Fig. 7. Site 5. Panoramic view of extreme northeast portion of Laguna Figueroa, August, 1977, after a wet spring and summer. Photo courtesy of B. Tibo.
Fig. 8. Close-up of the brown crust surface at site 5. Photo courtesy of B. Tibo.

Site 3. Well -laminated *Microcoleus* mat, as described by Horodyski et al. (1977, p. 683) with black firmer muds about 15 cm below the surface. Puddles on higher surfaces of the desiccation polygons (op. cit.) become deep orange in color (Fig. 6). In some places the surface had been disturbed, exposing black, odoriferous muds. Samples of this mud were the source of many subsequent anaerobic enrichments.

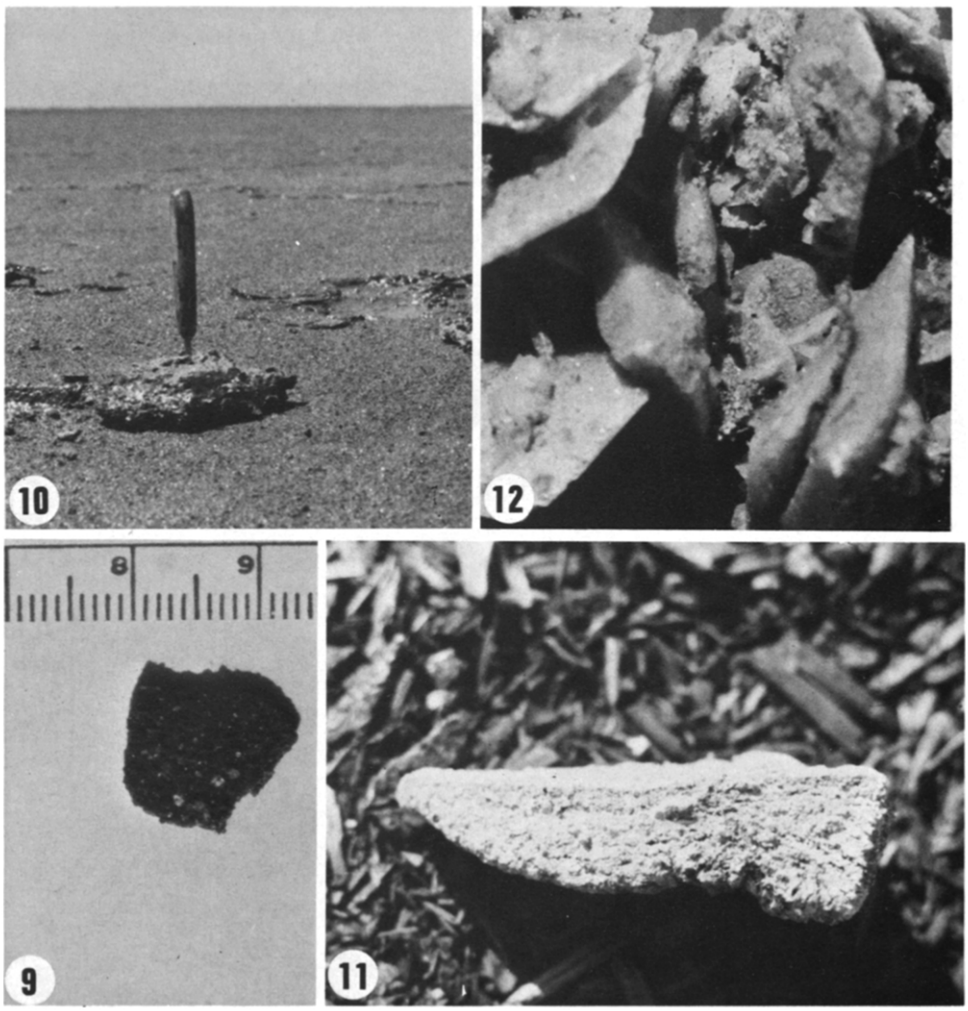


Fig. 9. Brown crust from surface at site 5, about 10^8 microbes per ml were estimated to reside on its surface.
Fig. 10. Panoramic view of site 5 mud.
Fig. 11. Section through desiccated *Microcoleus* mat sample 30 months after removal from the field, source of many microbial enrichment cultures.
Fig. 12. Rose gypsum crystal.

Site 4. Dark surface, vuggy, brecciated with centimeter level topography, random grey and white recrystalized halite and gypsum underlain by the *Microcoleus* mat, here called "brecciaform."

Site 5. Hard, crusted, brown surface with a much less steep topography — fractions of a millimeter. At places the crust is raised, giving evidence of gas beneath. Below the surface is black and brown sand. The photosynthetic microbial brightly colored community found at other sites is either poorly developed here or absent. This site is referred to as "brown crust" (Figs. 7—10).

Site 6. A channel in which the chlorophyte *Enteromorpha* was abundant. The edges of the channel are surrounded by a very extensive, cohesive, bright pink layer, here called "pink scum", which can be lifted in sheets as large as a square meter before ripping. Under it are flocculent black muds to depths of several decimeters.

FIELD OBSERVATIONS

Direct observations in the field were made with a Nikon field H microscope which provides magnification to about 1000 times. Some samples were collected from mats and muds and incubated under anaerobic conditions in Brewer jars into which packets of CO_2/N_2 were released (Gas Paks, BD, Co.). Many others were cut out of the laminated mats with a knife and simply wrapped in foil. For electron microscopy, we took samples of microbes bathed in sea water. These were placed directly into jars containing 2% glutaraldehyde in 0.1 M phosphate buffer made with water from the field. For scanning microscopy we took samples of fresh or dried mat which were placed directly on stabs, evacuated, and observed.

In addition to field observations, we conducted microscopic studies of collected samples for up to two weeks in laboratories of the Department of Geology, California Institute of Technology and the Sepulveda Veterans Administration Hospital. Mud sediment samples were either placed in about 5% formaldehyde or kept "fresh " in closed containers or were simply permitted to dry out. From time to time after returning to Boston we used both mud and dry samples as inocula into a variety of media. Because our treatment of the samples varied according to organism sought, we have included details of acquisition along with our discussion of each major type of organism recovered.

CULTURE MEDIA

For enrichment cultures of the heterotrophic anaerobes we incubated mud or subsurface mat samples at 30—37°C in Brewer jars as follows:

Enrichment medium (EM). KH_2PO_4, 0.05 g; NH_4Cl, 0.1 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; 10 ml of 100% lactic acid; yeast extract, 0.1 g; sodium

thioglycollate, 0.1 g; dissolved in 100 ml sterilized filtered sea water. Depending on the purpose, we added from zero to 1.5% agar to harden the medium.

Nitrogen-fixing medium (NFM). K_2HPO_4, 0.1 g; $MgSO_4 \cdot 7H_2O$, 0.02 g; $CaCO_3$, 0.05 g; $FeCl_3 \cdot 6H_2O$, 0.025 g; $NaMoO_4 \cdot 2H_2O$, 0.05 g to which 1 g sucrose was added to 100 ml artificial seawater. For slants and plates we added 1.0% Noble agar. To make artificial seawater we used the formula given by Aaronson (1970): NaCl, 2.348 g; Na_2SO_4, 0.392 g; $NaHCO_3$, 0.019 g; KCl, 0.066 g; KBr, 0.0096 g; H_3BO_3, 0.0026 g; $MgCl_2 6H_2O$, 1.061 g; $SrCl_2 \cdot 6H_2O$, 0.004 g; $CaCl_2 \cdot 2H_2O$, 0.1469 g; added to 100 ml distilled H_2O (initial pH was 7.2).

Photosynthetic bacteria enrichments. Here we used purple sulfur standard photosynthetic bacteria media (Aaronson, 1970). Closed jars of mud were returned to the labs and left for months illuminated by a 25 W incandescent bulb at a distance of 10—25 cm at room temperature. Samples were taken periodically.

DIVERSITY OF ORGANISMS

Prokaryotes

No matter the sample or the site, every observation revealed at least 10^8 — 10^9 microorganisms per milliliter. Even the solid hard crust (Fig. 9) and the gypsum rose desert crystal (Fig. 12) were covered with microbes. Dry fungal hyphae and spores could be seen and teased out easily, and a thin film of sea water would always bring microbes into view in, on, and around halite and gypsum crystals as well as diatom frustules, usually empty. In all field observations, small coccoids and bacilli (many motile) requiring oil immersion lenses for visualization, far outnumbered all other types or organisms, including cyanobacterial trichomes. For months after a field excursion, tiny quantities of sediment immersed in filtered or sterilized seawater still contained many distinct types of viable microbes. The inoculation of less than 1 mg dry sediment into enrichment media or photosynthetic bacterial media, including anaerobic media, incubated at 27—30° C always resulted in rapid growth of more types of microbes than can possibly be described here.

To reduce the staggering diversity to manageable proportions, we have limited our discussion of observed organisms that were conspicuous in the field, morphologically distinctive, easily grown in the initial anaerobic inoculations and subsequently isolated into either pure or highly enriched cultures, or those that repeatedly grew from desiccated mat samples up to 30 months old (Fig. 11).

The most frequently enriched forms — tiny vibrios, rods, and cocci — developed within several days and were seen observed in all samples moistened with sea water. Non-notile, motile cocci and rods were extremely conspicuous in all samples grown anaerobically in heterotrophic medium (Fig.

13). At least one of these unicells — a facultatively aerobic, polarly flagellated, salt-requiring microbe measuring 1.4 × 2 μm — has been identified as a member of the genus *Beneckea*. It produces gas fermentatively, reduces nitrate to nitrite during anaerobic respiration, and forms the bright red pigment, prodigiosin (Giovannoni, 1979, 1980). Thus it was closely related to *Beneckea gazogenes*, which was isolated by Harwood (1978) from Sippewissett, MA, salt marsh mud. We have not been able to assign to genera many of the other small unicells observed during these studies and have therefore not included any in the following discussion.

Blue green bacteria. In addition to *Microcoleus*, *Lyngbya*, and *Entophysalis*, which are well described by Horodyski and his colleagues, we frequently saw several species of *Phormidium*, *Pseudoanabaena*, *Schizothrix*, and *Anabeana*. These cyanophytes, kindly identified by S. Golubic, developed as blooms in mats older than 20 months to which sterile sea water had been added. Their sheaths were always covered with bacterial blooms as well.

Spirochetes. We observed several morphologically distinct organisms, thought to be spirochetes, in mat and mud samples. Two, designated BA-1 and BA-2, were isolated from fresh mud samples collected at sites 3 and 6 and are now maintained in pure culture. They were first obtained on enrichment medium in broth and were subsequently purified using the techniques of Canale-Parola (1977). In the laboratory, samples from EM cultures containing spirochetes were placed on well plates containing 0.5 g glucose, 0.2 g peptone, 0.1 g yeast extract, 0.1 g sodium thioglycolate added to 100 ml seawater, and 0.15% (w/v) agar. After four days at room temperature the spirochetes were observed to have migrated through the agar and were obtained in pure culture by removing samples from the periphery of the resulting subsurface veil and serially diluting them in tubes of isolation medium containing 1% (w/v) agar. Single colonies observed after four days incubation at 30°C were removed and inoculated into this isolation medium plus 0.15% agar. The two distinct spirochetes grow well on this medium with or without agar (Fig. 14, 15). Both are obligate anaerobes with a requirement for NaCl, demonstrating they are marine organisms. BA-1 is loosely and irregularly coiled and remains viable in broth for a maximum of only about 60 hours after inoculation; BA-2 is tightly coiled and remains viable for several weeks. Another difference of particular interest in this study is preliminary immunofluorescence and gel electrophoretic evidence for the presence of microtubule protein (tubulin) only in BA-2 (Margulis et al., 1978). Electron microscopy studies have confirmed that BA-1 displays the periplasmic ultrastructural features common to spirochetes of the genus *Spirochaeta* Ba-2 (EM's courtesy of G. Cooper).

Nonphotosynthetic spirilli. Spirilli abounded in all fresh mud samples (Fig. 16). Some very long ones, designated Sp-1, were mistaken at first for spirochetes. These could be enriched by three days of anaerobic growth in the EM at a pH adjusted to 6.1 and incubation at 30—37°C. They also grew

aerobically, but we could not assess the true nature of their relationship to oxygen because even the highly enriched cultures were always contaminated by other tiny microbes. Sp-1 are highly motile and attain great lengths, ranging from 8.3 to 18.3 μm; their wavelengths range between 2.5 and 5.0 μm

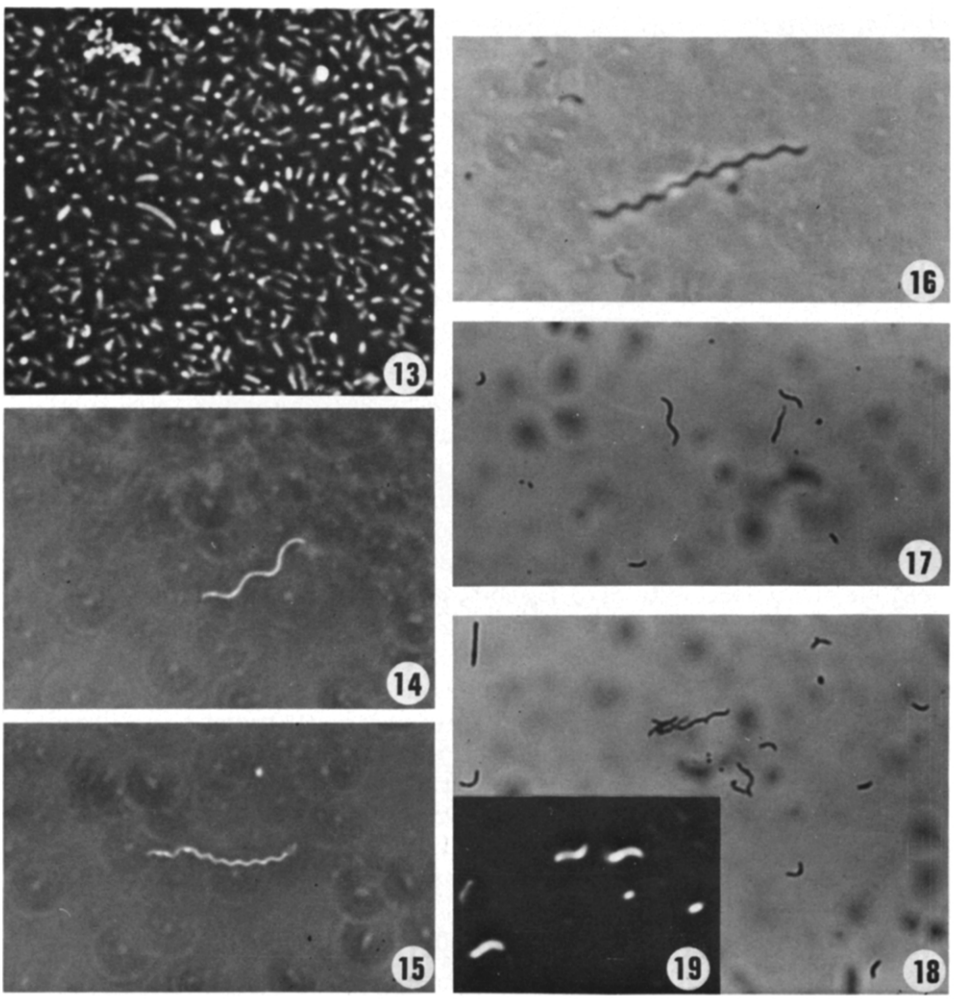


Fig. 13. Non-motile and motile coccoids and rods; the dominant life form other than the surface cyanobacteria at Laguna Figueroa.

Fig. 14. Microbial mat spirochete, Ba-1. (× 1000)

Fig. 15. Microbial mat spirochete, Ba-2. (× 1000)

Fig. 16. Microbial mat spirilli in enrichment cultures: Sp-1 live. (× 1000)

Fig. 17. Microbial mat spirilli in enrichment cultures: Sp-2 live. (× 1000)

Fig. 18. Microbial mat spirilli in enrichment cultures: Sp-2 fixed in 45% acetic acid. (× 1000)

Fig. 19. Anaerobic microbial mat spirilli in pure culture: Sp-3 live. (× 1000)

and their amplitudes between 0.8 and 1.6 μm. They travel at a velocity of about 18 μm per sec. That these spirilli bear polar flagella at each end of the cell was confirmed by negative staining as well as by transmission and scanning electron microscopy performed on samples from enrichment medium (Figs. 16—22).

Two other morphologically distinctive spirilli, Sp-2 and Sp-3, were consistently seen in both fresh and enrichment culture samples. Sp-2 is the smaller of the two organisms, but may also develop up to over three wavelengths (Figs. 17, 18, 20). This organism was enriched in EM from which yeast extract and NH_4Cl had been removed. After 3 days incubation at 30°C, pH 6.1 in this medium in air, Sp-2 formed a subsurface band in tubes or migrated below the surface in soft agar; thus, we believe they are probably microaerophilic. They were fixed in 45% acetic acid on slides, retaining their morphology so that measurements could be made (Figs. 18, 21, 22). These are from 8.3 to 10.6 μm in length, 3.32 μm in wavelength, and 0.8 μm in amplitude. In healthy cultures they also swim at a velocity of about 18 μm per second.

We easily distinguished Sp-3 by its morphology and growth characteristics (Figs. 19, 20). It is short and thick (length 4.2—14.9 μm, width 1.7—2.5 μm). It developed only a few wavelengths (3.3—8.3 μm; amplitude, 2.5—5.0 μm), but moved at high velocity: 28 μm per sec. Sp-3 grew optimally at higher initial pH (7.7) in a calcium lactate enrichment medium to which asparagine had been added. At 0.8% agar it formed cloudy white, individual colonies deep in the agar (Fig. 23). Unlike Sp-1 and Sp-2, Sp-3 penetrated hard agar (1.5%) and migrated through it, developing at depth. It did not grow under aerobic conditions. The ability of Sp-3 to penetrate hard agar probably reflects the gelatinous viscosity of the *Microcoleus* mat samples from which they were taken.

All three spirilli are gram negative and were seen in samples from sites 3 and 6. No well characterized spirilli with these combinations of traits, especial ly none capable of anaerobic growth is known (Buchanan and Gibbons, 1974; Krieg, 1976); it is likely that further work will show at least the thick anaerobic spirillas are members of a new species.

Nocardia sp. In enrichment media we frequently saw filamentous microbes even when all sources of nitrogen were omitted from the sea water medium, colonies with gram variable cells developed either in broth or on plates with a distinctive morphology, recognized to be that of the genus *Nocardia* (Figs. 24—28). They produced acid-fast spores, beginning as clubs that varied from 1.7 to 2.5 μm in diameter. The diameter of the filaments varied from 0.8 to 1.7 μm at the hyphal tips. The length of the spores ranged between 1.7 and 3.3 μm. These organisms developed well on a medium designed for nitrogen-fixing bacteria — one that did not support the growth of the two strains of *Beneckea* unless nitrogenous compounds were added. Attempts to verify the fixation of atmospheric nitrogen by this species of *Nocardia* are underway. These organisms do not grow at all if NaCl is omitted from the medium or is replaced by KCl. They grow poorly at 1.5% NaCl, more

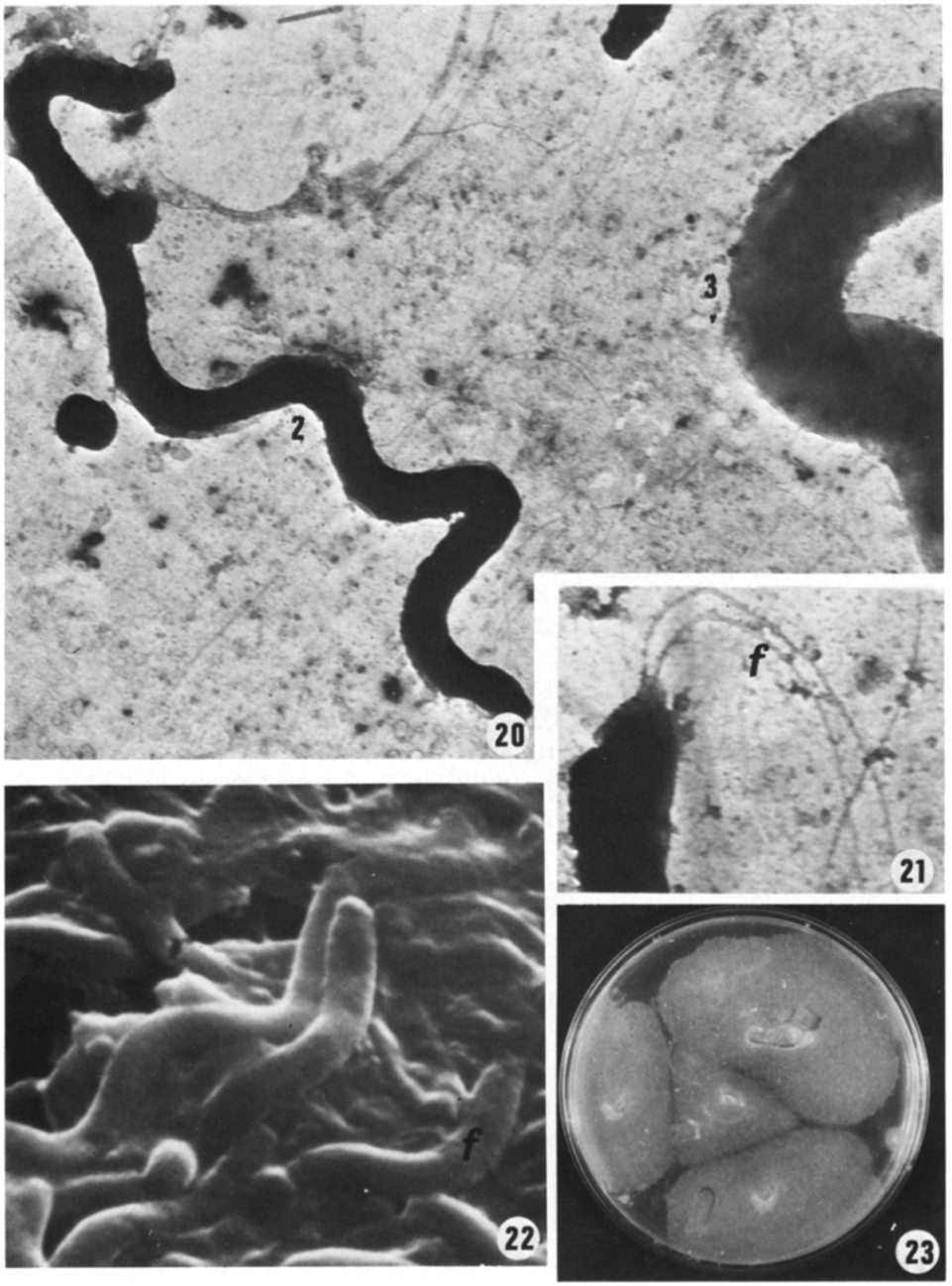


Fig. 20. Electron micrograph, negative staining of spirillum Sp-2 (2) and Sp-3 (3). Preparation and photo courtesy of Dr. L.P. To.
Fig. 21. Electron micrograph, negative of one end of spirillum Sp-2 (f = flagellum). Preparation and photo courtesy of Dr. L.P. To.
Fig. 22. Scanning electron micrograph of spirillum Sp-2 (f = flagellum).
Fig. 23. The anaerobic microbial mat spirillum, Sp-3, colonies on hard agar.

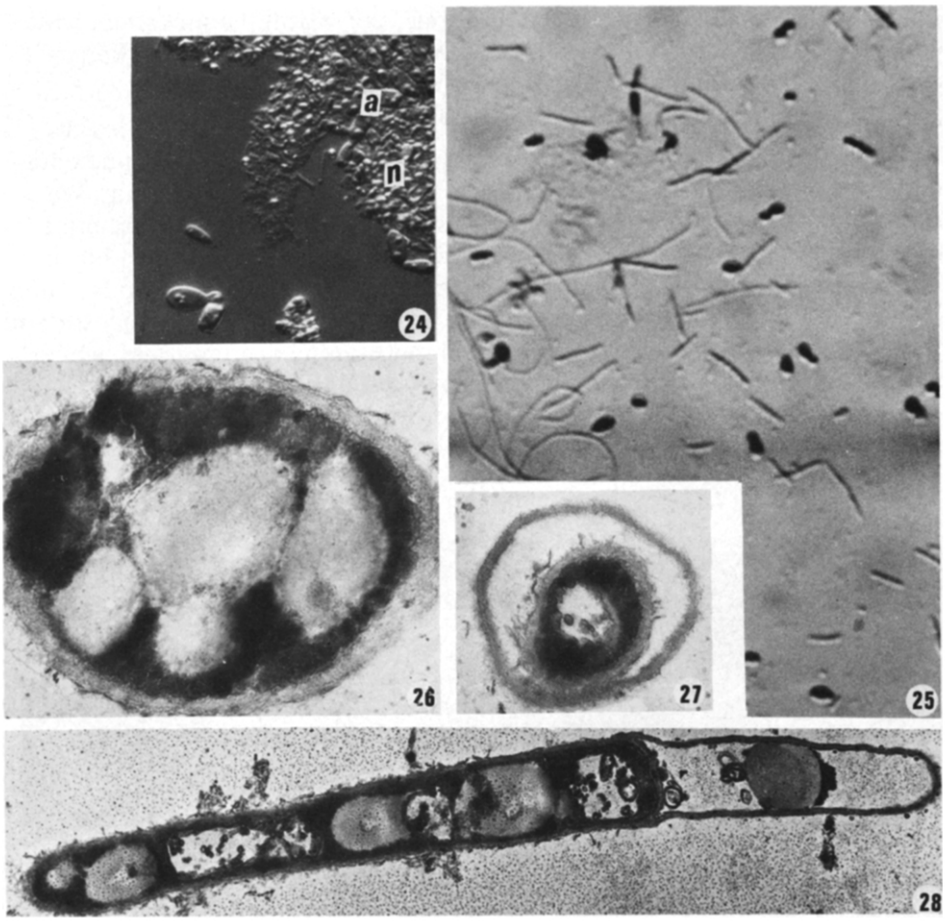


Fig. 24. *Nocardia* (n) from live colonies on agar showing its association with *Aspergillus sydowi* (a). (Nomarski phase contrast optics, × 100)
Fig. 25. *Nocardia*, gram stain preparation. Note gram positive club heads of actinospores (Nomarski phase contrast × 2000)
Fig. 26. Transmission electron micrograph of hyphae of *Nocardia*, longitudinal section through filament. (× 18,000)
Fig. 27. Transmission electron micrograph of actinospore of *Nocardia*. (× 45,000)
Fig. 28. Transmission electron micrograph through filament of *Nocardia*. (× 45,000)

vigorously as the salt concentration is increased. Salt crystals precipitating out in the medium seem not to harm them at all. We have now reisolated these organisms several times from gypsum crystals (Fig. 29) and from hard dry mat over 18 months old (Fig. 11) both containing crystalline NaCl. They showed the most rapid vegetative hyphal growth at 10% NaCl. At sea water concentrations they were present primarily in the form of spores (figs. 25, 27) We often isolated *Nocardia* from or found them associated with the salt requiring *Aspergillus* fungus described below. Isolated into pure culture with

shake tubes using 1% agar and serial dilutions, they form distinct small white colonies. Since no marine nitrogen-fixing *Nocardia* are described in Bergey's Manual, it is likely that this organism also represents a new Species.

Sulfate-reducing bacteria. The odor of hydrogen sulfide, usually indicative of sulfate reduction by microbial members of the marine sulfur-cycling community, was easily detected as one walked across the *Microcoleus* mat. We isolated three morphologically distinct sulfate reducers from mat and mud samples collected at sites 3 and 6. They were initially identified by a black precipitation of iron sulfide in anaerobic enrichment cultures (Postgate, 1963) and purified by repeated transfers. Sulfate served as the electron acceptor and lactate as the electron donor. Cysteine and methionine decomposers, which would also produce H_2S, were checked for by plating on nutrient — McConkey and glucose peptone agars. A nonsporeforming, gram negative, motile spirillum (measuring 0.4 μm × 1 to 5 μm) and an anaerobic rod measuring

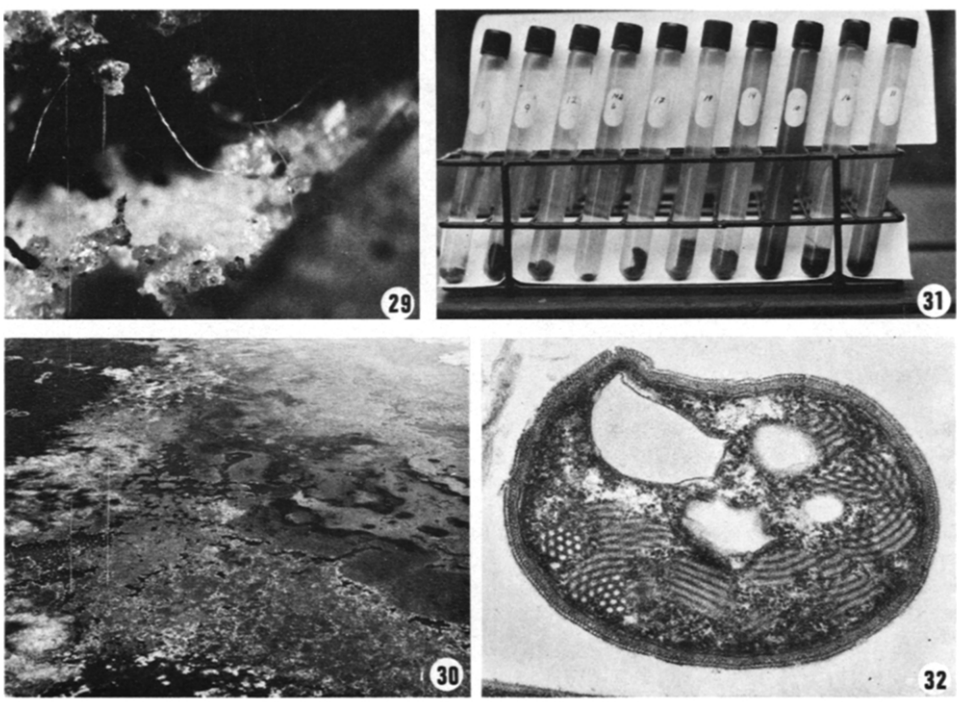


Fig. 29. Gypsum crystal, low power light micrograph showing thread that contains *Nocardia* and *Aspergillus*. Same crystal as Fig. 12 (× 10)

Fig. 30. Purple photosynthetic bacteria as surface component of the microbial community at site 6. Photo courtesy of S. Awramik.

Fig. 31. Photosynthetic bacteria in enrichment media. Some were identified as chromatia or rhodopseudomonads on the basis of morphology.

Fig. 32. Unidentified photosynthetic purple sulfur bacterium taken from water samples at site 6. Note distinctive thylakoids. Transmission electron micrograph. (× 50,000)

0.6 μm × 1 to 5 μm have been tentatively identified as belonging to *Desulfovibrio* species. Both utilize lactate and pyruvate as carbon sources and contain c_3 cytochromes and desulfoviridin. For cytochrome c_3 demonstration we concentrated fully grown cultures by centrifugation and examined them by spectrophotometry after addition of the reducing agent, sodium dithionate. Strong absorption bands at 525 and at 553 nm indicated the presence of cytochrome c_3. Desulfoviridin was tested for by the addition of 1*N* NaOH, which releases the chromatophores of the pigment. It is detected by a pink fluorescence under ultraviolet light (Postgate, 1959) and a characteristic absorption at 630 nm. The third isolate, similar in morphology to that of Jones (1971), is a gram negative, nonsporeforming, anaerobic rod, 10—15 μm long that contains desulfoviridin and a c-type cytochrome. More thorough investigation of the sulfate-reducing communities at Laguna Figueroa, as well as other diagnostic test results will be discussed in future papers.

Photosynthetic bacteria. We could readily observe photosynthetic bacteria in brightly colored layers of pinks and reds just beneath the cyanophytes. In

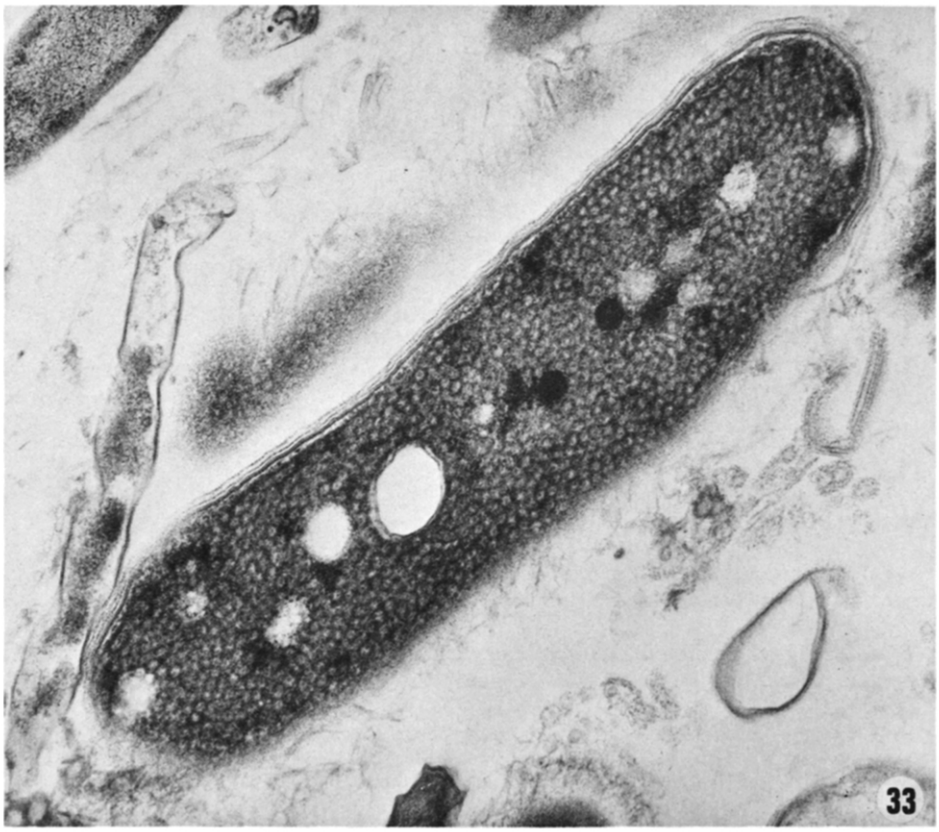
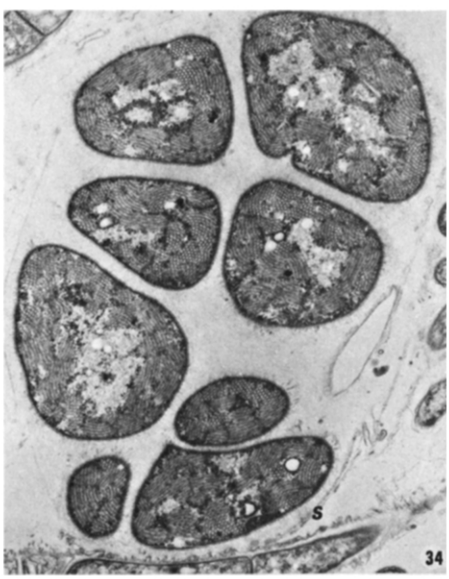


Fig. 33. A second unidentified purple photosynthetic sulfer bacteria from site 6. Transmission electron micrograph (× 50,000)

some cases (for example, at site 6) purple sulfur bacteria with probable affinities to *Thiocapsa* (Buchanan and Gibbons, 1974) form extensive scums (Fig. 30). Light microscopic observations revealed that the scum is filled with yellow globules, interpreted to be sulfur granules, left as holes in the transmission electron micrographs (Fig. 32). Two distinctive features of the organism are the peculiar sixfold symmetry of the well developed thylakoids, its presence as either a single cell or in extensive sheets embedded in sheath. In our samples this photosynthetic bacterium was also associated with a second morphologically distinctive photosynthetic coccoid (Fig. 33). Preliminary experiments with autotrophic media incubated either anaerobically or with reducing agents in the light indicate that in addition to these scum-dwellers a great variety of photosynthesizers are present in both mat and mud samples (Figs. 31, 34, 35). In addition to the *Thiocapsa*-like microbe, several well-known genera such as *Rhodospirillum* sp. and *Chromatium* sp. were observed. The isolation and characterization of the easily enrichable photosynthetic bacteria will be the subject of subsequent studies.



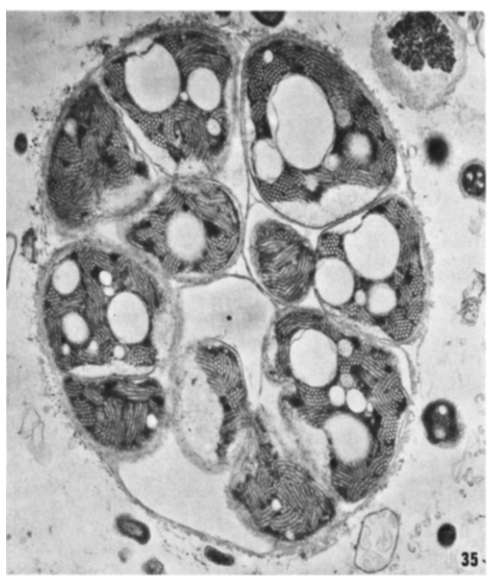


Fig. 34. *Thiocapsa* sp? one of the major surface microbes at site 6. Note the association between a different bacterium and the sheath (s). Transmission electron micrograph. (× 25,000)
Fig. 35. *Thiocapsa* sp? same sheathed photosynthetic microbe as in Fig. 34. Note holes that remain after intracellular deposits fall out. Transmission electron micrograph. (× 25,000)

Miscellaneous bacteria. We frequently saw long filamentous forms and septate cocci (Figs. 36, 37) in live preparations and micrographs of material taken from the field. The absence of well developed thylakoids and the

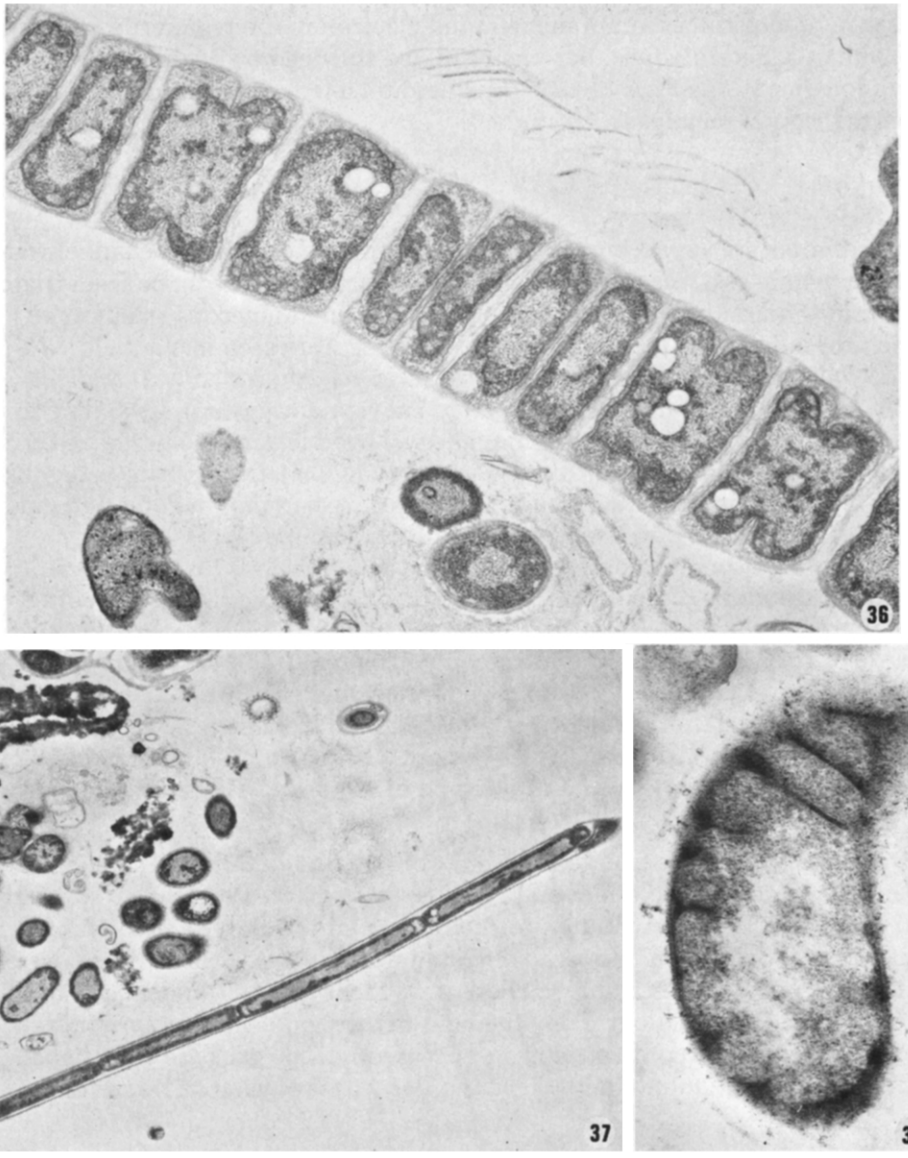


Fig. 36. Unidentified distinctive filamentous microorganisms probably not cyanobacteria because they lack thylakoids. Transmission electron micrograph. (× 37,5000)
Fig. 37. Unidentified distinctive filamentous microorganism. Transmission electron micrograph. (× 17,500)
Fig. 38. Unidentified distinctive segmented filamentous microorganism. Transmission electron micrograph, courtesy of Dr. L.P. To. (× 25,000)

presence of a distinct membranebounded electron-dense region suggests these filaments are heterotrophs, but until isolated they can be distinguished only morphologically. As Figs. 38, 39, 53 illustrate, much of the microbial diversity at the lagoon remains to be studied.

Eukaryotes

Eukaryotes are very scarce at Laguna Figueroa except in the channel waters of site 6 where fish, macroscopic algae, and aquatic plants can be seen. Diatom tests are common but living diatoms rare. We made numerous attempts to enrich for eukaryotic microbes since only a few were seen in the field. We found only two. One, a microplanktonic alga, was identified as *Dunaliella salina* on the basis of its morphology and encystment pattern, its ability to grow in standard *Dunaliella* medium, and the production of a bright red pigment, presumably hematochrome, when growing on the evaporite polygons at site 2. The other is the salt-requiring fungus, *Aspergillus sydowi*, the only eukaryotic organism consistently and easily enrichable from nearly any sample. In addition, populations of amoeba and a curious unpigmented *Chrysarachnion*-like organism (Fig. 40) were frequently observed in enrichment cultures for photoheterotrophs.

Except for *Dunaliella* and these amoebae, many attempts to grow protists from mat material failed. Occasionally we saw ciliates in fresh mat material, however, they were not present with high enough frequency to be studied, and none could be enriched. The only metazoans seen in hours of observation were many specimens of a single type of copepod! The paucity of eukaryotes was impressive.

Dunaliella salina. We isolated this chlorophytic halophilic *Dunaliella*, from both fresh three-week-old mat and from severely desiccated mat up to nearly two years old. They contributed to the redness of the water at site 1 and were a major component of the microbial community on the surface of the desiccation polygons of sites 2 and 3. These were identified as *Dunaliella salina* on the basis of morphology using the light microscope and on the ultrastructure level using transmission electron microscopy. The cells are tear shaped with homokont undulipodia (eukaryotic flagella), a single plastid in the

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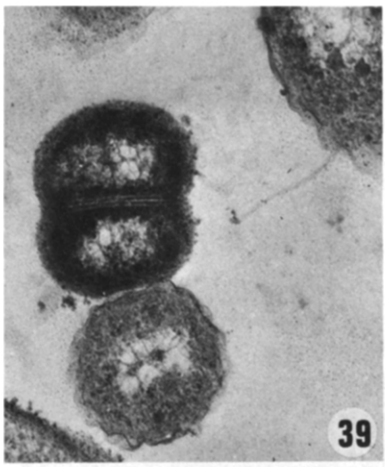
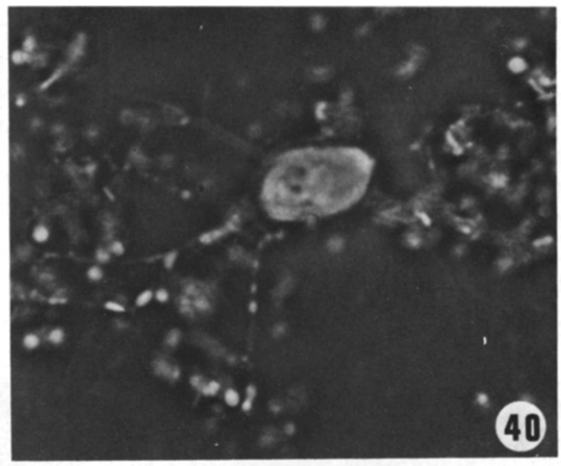
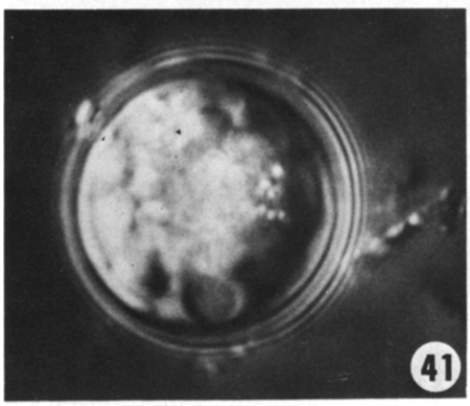
Fig. 39. Unidentified coccoids common in mat samples, site 6. Transmission electron micrograph, courtesy of Dr. L.P. To. (× 14,300)
Fig. 40. *Chrysarachnion*-like protist found in abundance for a few days in bloom developing from desiccated mat samples rewet with sterile seawater, live. (× 1000)
Fig. 41. *Dunaliella*, light micrograph of cyst. (× 100)
Fig. 42. *Dunaliella*, transmission electron micrograph of vegetative cell; e, eyespot; g, golgi; n, nucleus; m, mitochondria. (× 14,300)
Fig. 43. *Dunaliella*, transmission electron micrograph of plastid, showing distribution of starch in pyrenoids. (× 21,500)
Fig. 44. *Dunaliella*, transmission electron micrograph of kinetosomes and undulipodia showing typical volvocalean arrangement. (× 30,000)

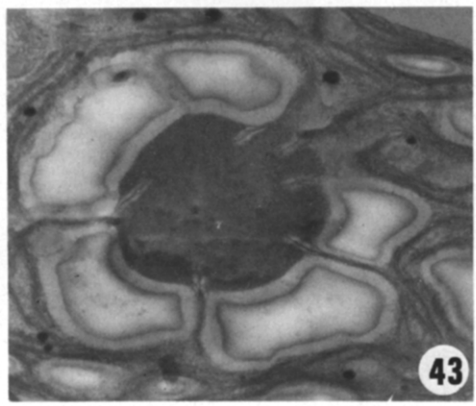
posterior half of the cell, and a single nucleus in the anterior portion (Figs. 42, 44). The plastid contains a central pyrenoid (Fig. 43). There are starch granules around the pyrenoid and within the thylakoids. Each cell contains a conspicuous eyespot but no cell wall.

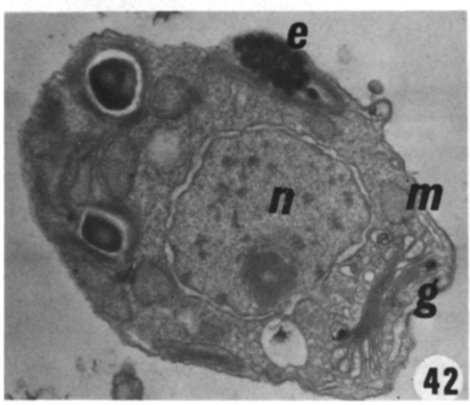
Our samples revealed the presence of these protists at three stages: a flagel-

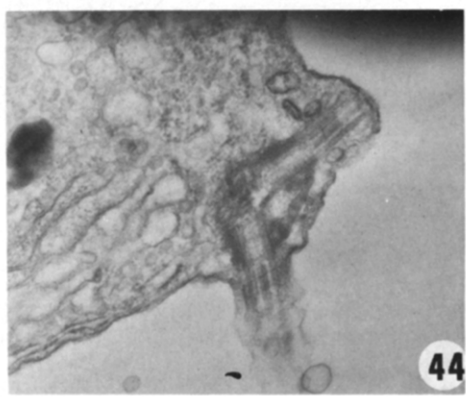












lated motile stage (Fig. 42), a spherical nonmotile stage, corresponding to the flagellated cells with respect to internal morphology; and an encysted stage (Fig. 41). As the cells begin to encyst they appear to lose their tear shape, becoming flagellated spheres. The motile forms range in diameter from about 6 to 10 μm, while the diameters of the nonmotile spheres and of the large cysts are about 10 and 15 μm, respectively. The large cysts differ from the motile and vegetative forms by the presence of a thick cell wall surrounding granular cytoplasm and large vacuoles (Fig. 41). Originally green in color, the cysts become reddish-brown with maturity. Motile forms of *Dunaliella* are bright red owing to their production of hematochrome at high light intensities (Loeblich, 1962); however, although we saw strikingly red *Dunaliella* in abundance in the field these flagellates grown in the laboratory were green.

Dunaliella remained motile if the salt concentration in the media exceeded that of sea water. When salinity was lowered to 3.4% they formed cysts. The desiccated mat samples contained many large cysts that developed into

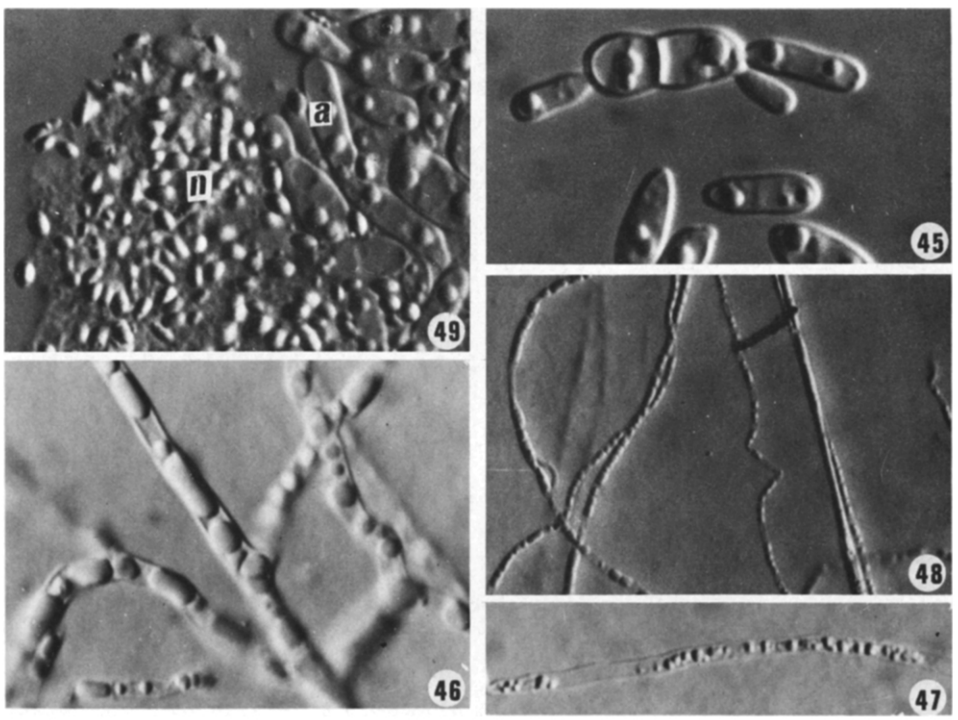


Fig. 45. *Aspergillus sydowi*, microscopic morphology as a function of salinity on nitrogen-free medium, 3.5% NaCl. (× 1000)
Fig. 46. *Aspergillus sydowi*, 4.5% NaCl. (× 1000)
Fig. 47. *Aspergillus sydowi*, 5.5% NaCl. (× 1000)
Fig. 48. *Aspergillus sydowi*, 10% NaCl. (× 1000)
Fig. 49. *Aspergillus sydowi*, light micrograph showing association of actinospores (n) with the vegetative *Nocardia* sp. actinomycete (a), 3.5% salt. (× 1000)

motile forms when cultured. Experiments with slow evaporation of enrichment cultures in high saline media indicated that cyst formation can also be induced by desiccation. The alternating dry and wet periods characteristic of Laguna Figueroa have selected for adaptations to hypersaline rather than marine conditions. Apparently cyst formation with desiccation or with salinity less than 0.6 *M* or more than 4.0 *M* NaCl is the key to the survival of this *Dunaliella* strain in the hypersaline lagoon.

Aspergillus sydowi. In our samples, this organism was regularly associated with *Nocardia*, except at 10% NaCl. Both *Aspergillus* and *Nocardia* require sodium chloride for growth, but their morphology varies as function of NaCl concentration, as shown in Table I.

Aspergillus sydowi was kindly identified by Dr. Philip B. Mislivec of the Division of Microbiology, Food and Drug Administration, Washington, DC, on the basis of its morphology on Czapek's and malt agar (Thom and Raper, 1945). This species, which is considered xerophytic, is known to grow at relative humidities as low as 78% and has hithertofore been known primarily as a common contaminant of smoked meat. It grows optimally at NaCl concentrations from 3.5 to 4.5%; it can survive at higher concentrations but undergoes distinct morphological alteration (Figs. 45—49). At salt concentration higher than 5.5% the conidia do not germinate; at 1.5% only hyphae are present; at sea-water salinities, fruiting bodies are formed regularly (Fig. 50). This fungus clearly has low nitrogen requirements for growth, and as long as *Nocardia* is also present, it will grow vigorously in a nitrogen-free medium.

A clue to its relationship with *Nocardia* can be seen in Fig. 51. Often *Nocardia* spores are attached to the fungal conidiospores, insuring the co-dissemination of these two species. We have seen spores sticking to dried cyanophytan trichomes and sheaths. (Both actinospores and conidia germinate soon after placing them on nitrogen-free medium). Both of these filamentous organisms tolerate extreme desiccation and high salinity, and their spores remain viable up to 3 months, at least.

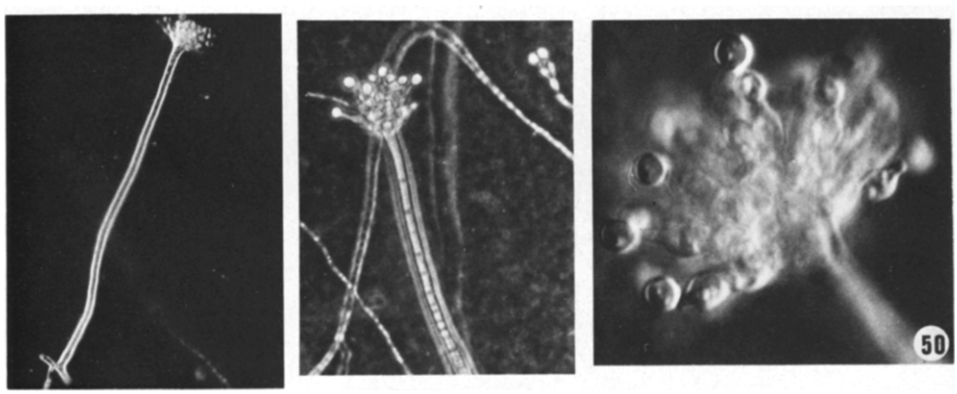


Fig. 50. Conidiophores of *Aspergillus sydowi* grown in nitrogen-free medium in 5.5% NaCl. Left, entire mature conidiophore (× 100); center, conidiophore bearing conidia and maturing conidia (× 400); right, mature conidia, live (× 1000).

TABLE I

Nocardia—Aspergillus association: morphology and growth as a function of salt concentration*

	NaCl concentration (%)					
	0	1.5	3.5	5.5	7.5	10
Nocardia sp.	no growth	slow growth thin gram negative hyphae, terminal gram positive club heads	good growth, abundant actinospores, primarily reproductive structures	optimal growth, primarily vegetative hyphae, few actinospores	good growth Primarily long skinny branched vegetative hyphae, few antinospores	good growth
Aspergillus sydowi	no growth	very slow growth, branched hyphae	optimal growth, conidia, conidiophores, broad hyphae	optimal growth, conidia, conidiophores, broad hyphae	slower growth thinner granulated hyphae, very few conidia	growth very slow, extremely thin hyphae full of conspicuous granules

*Observations made at 7, 14 and 21 days, agar, see Figs. 45—51. N_2 fixing medium, see Methods.

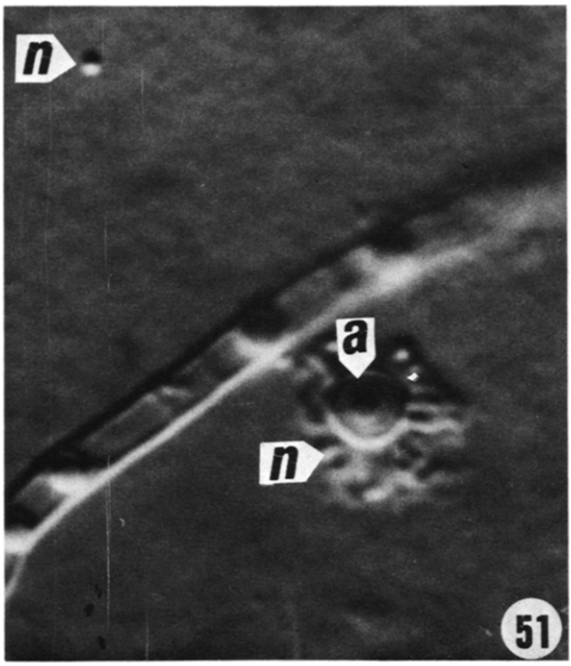
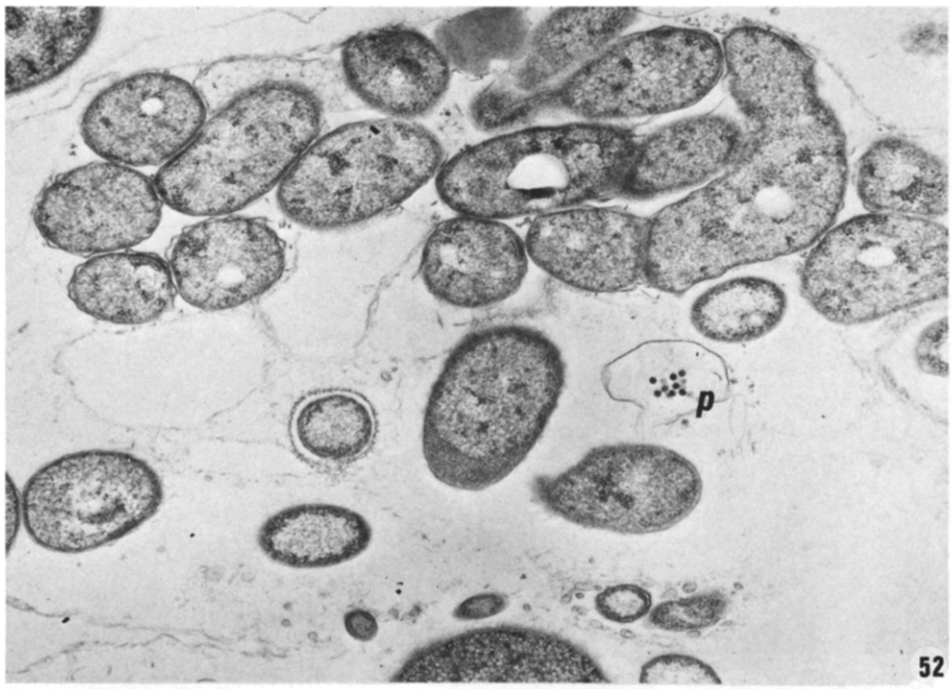


Fig. 51. Association of actinospores of *Nocardia* sp. (n) with mature conidium of *Aspergillus sydowi* (a), live. (Nomarski phase contrast, × 1000)

DISCUSSION

Knoll and Barghoorn (1977) interpreted the Fig Tree spheroids observed in petrographic thin sections to be microfossils of prokaryotic organisms in cell division. These microfossils were found in black cherts that came from the same stratigraphic horizon in the Barberton mountainland as did the banded chert sample (Fig. 1), which suggests that our comparison between the laminated mats at Baja California and the Swartkoppie carbon-rich sediments may be valid. It is likely that the detailed comparison we are attempting between the anaerobic microbes of the microbial mats at Baja and these Archean microfossils and sediments will yield insight into anaerobic microbial communities thought to have evolved during the lower Precambrian. Although there is no proof that the Swartkoppie chert formed in a hypersaline lagoon, it is certainly possible. The ability of modern prokaryotic communities to thrive under hypersaline conditions is a factor permitting them to persist and dominate an extreme environment in a world that today is dominated by eukaryotes.

In samples taken from the anaerobic zone of the hypersaline Laguna Figueroa, we were able to study about fifteen physiologically and morphologically distinctive microorganisms. In addition we observed bacteriophage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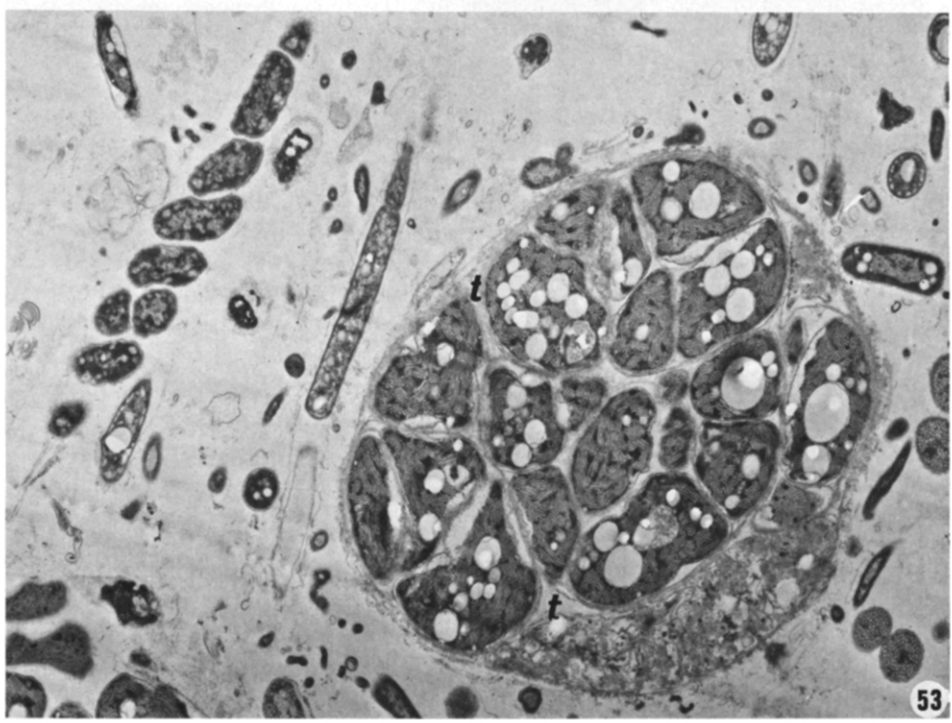
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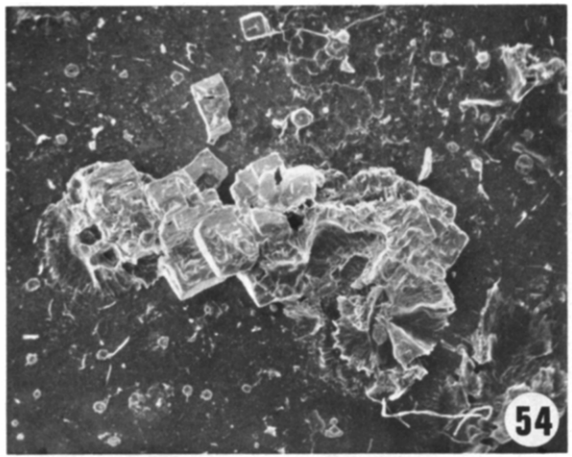
and a wide diversity of unidentified microbes (Figs. 52, 53). However, many more types were present in the carbon-rich layers of sediment taken from these sites. Even in the foamy waters of site 2 and the harder dryer brecciaform or crusty surfaces of sites 4 and 5, it was easy to observe an estimated 10^7—10^9 microbes per ml, predominantly unicellular bacteria. Beneath the low flat mat dominated by the cyanobacterium *Microcoleus* are muds and scums rich in anaerobic photosynthetic bacteria; these communities of microbes are clearly stratified according to environmental gradients and temporal cycles that are not yet entirely understood. They are closely associated with at least three types of evaporite minerals: gypsum, calcite and halite (Figs. 54—56). The question raised by our studies is: Would the compaction and silicification of comparable shallow-water sediments formed 3400 Ma ago lead to the sort of lithology that characterizes the laminated carbon-rich cherts of the Swartkoppie Zone as shown in Fig. 1? It is obvious that in the absence of well-developed stromatolitic laminae, layered communities of microorganisms that exhibit a great deal of diversity still prevail in certain habitats today. What is the fossil fate of such communities? An answer to this question would certainly be an advance in interpreting the meager fossil record of the Archean.

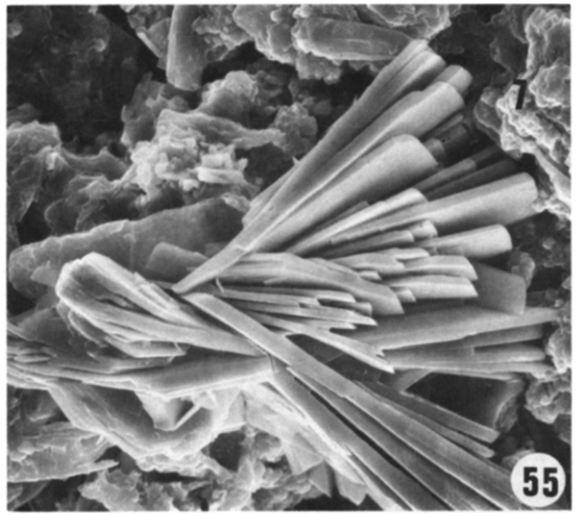
The microscopic comparison of the "typical" bacteria in carbon-rich laminae of live mat (sites 1—5) and a thin section of the ancient Swartkoppe chert is shown in Fig. 2. Is it possible that the dark tiny structures so abundant in the banded regions of the dark chert (Fig. 57—59) are fossil bacteria? What sorts of criteria might be used to verify the hypothesis of comparability of these sediments separated by 3400 million years in time? By further detailed studies of the micromorphology and magnetic properties as well as the analysis of many major and trace elements, for example, we hope to corroborate or invalidate this comparison.

Recent geobiological work [e.g., Monty (1973), Walter (1977), Golubic and Campbell (1979)] has reemphasized the concept that understanding the microbial contribution to the chemistry and geomorphology of the sediment is a prerequisite to the interpretation of the Precambrian. The fact that bacteria are less conspicious does not make them less important. The preliminary work described in this paper merely points up the complexity of an ecological zone that both ecologists and paleontologists have tended to ignore. The uncanny resemblance of our field site to an environment hypothesized essential for the formation of stratiform copper, silver, zinc and other metalliferous deposits (Renfro, 1974, fig. 5A) makes such bacterial studies of more than academic interest. Further knowledge of anaerobic microbial ecology, beginning with species recognition and identification and proceeding through physiological and *in situ* ecological analyses, is a prerequisite to the interpretation of sediments in general and of the carbon, iron and sulfur-rich rocks of the Archean in particular.

Fig. 52. Unidentified microbes and bacteriophage (p) from waters dominated by purple photosynthetic bacteria. Transmission electron micrograph. (× 25,000)

Fig. 53. Community of unidentified microbes associated with the sheaths of *Thiocapsa*-like photosynthetic microbe (t). Transmission electron micrograph. (× 11,250)





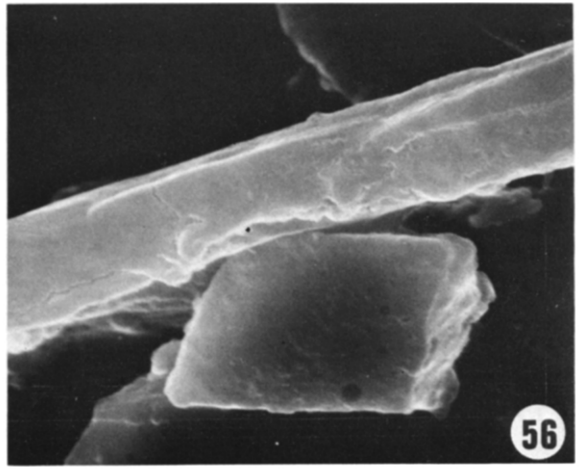
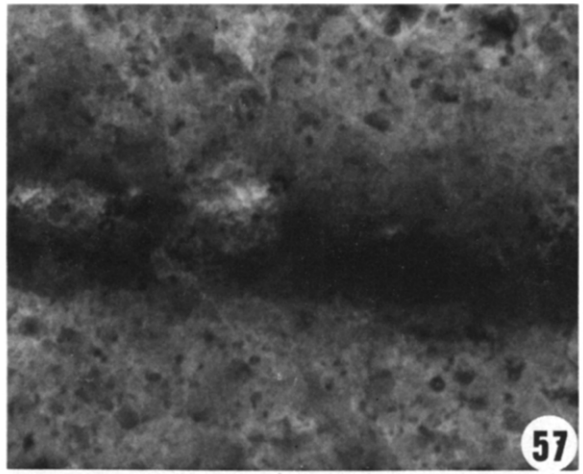
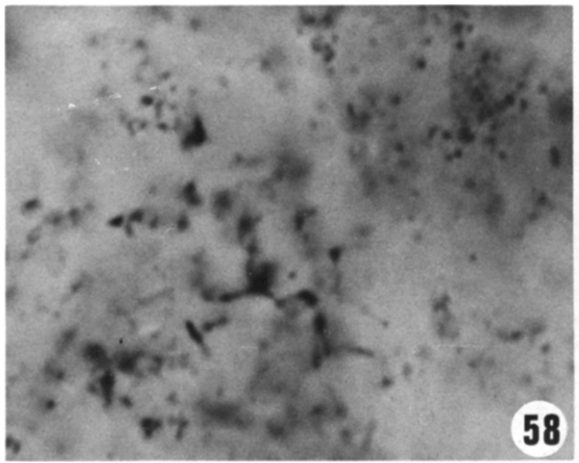
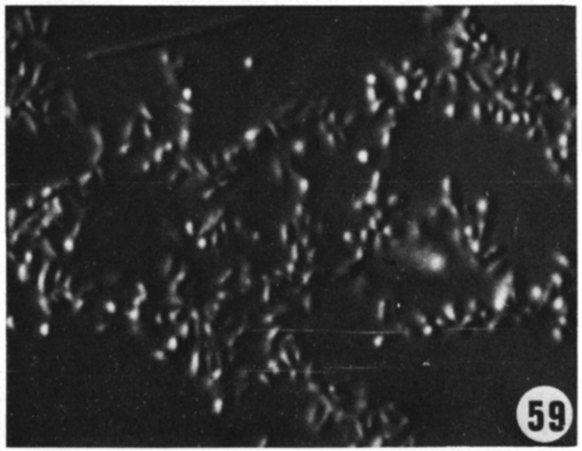


Fig. 54-56. Associations of microbes (all from dry *Microcoleus* mat) with major evaporitic minerals.
Fig. 54. Mat sample showing sodium chloride crystals, microbes seen to be abundant at higher magnification, scanning electron micrograph. (× 20)
Fig. 55. Amorphous material around gypsum crystals are associated with microbes, scanning electron micrograph. (× 1000)
Fig. 56. Calcite crystal associated with unidentified filamentous microbe, scanning electron micrograph (× 10,000)

Fig. 57—59. Comparison of the dominant members of the live microbial community of Baja with the thin section of Swartkoppie chert. Photos taken under comparable conditions with same Nomarski phase contrast microscope.
Fig. 57. Carbon-rich lamella from chert at low magnification. (× 100)
Fig. 58. Carbon-rich lamella from chert at high magnification showing prevalent dark spots in bands. (× 1000)
Fig. 59. Microbes from carbon-rich lamellae taken of the microbial mat some 2 cm below the surface. (× 1000)







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